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DOI: https://doi.org/10.1093/brain/awt322

Brain Advance Access published November 25, 2013

BRAIN
A JOURNAL OF NEUROLOGY

Brain volumes predict neurodevelopment in adolescents after surgery for congenital heart disease

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Patients with complex congenital heart disease are at risk for neurodevelopmental impairments. Evidence suggests that brain maturation can be delayed and pre- and postoperative brain injury may occur, and there is limited information on the long-term effect of congenital heart disease on brain development and function in adolescent patients. At a mean age of 13.8 years, 39 adolescent survivors of childhood cardiopulmonary bypass surgery with no structural brain lesions evident through conventional cerebral magnetic resonance imaging and 32 healthy control subjects underwent extensive neurodevelopmental assessment and cerebral magnetic resonance imaging. Cerebral scans were analysed quantitatively using surface-based and voxel-based morphometry. Compared with control subjects, patients had lower total brain ($P = 0.003$), white matter ($P = 0.004$) and cortical grey matter ($P = 0.005$) volumes, whereas cerebrospinal fluid volumes were not different. Regional brain volume reduction ranged from 5.3% (cortical grey matter) to 11% (corpus callosum). Adolescents with cyanotic heart disease showed more brain volume loss than those with acyanotic heart disease, particularly in the white matter, thalami, hippocampi and corpus callosum (all P-values < 0.05). Brain volume reduction correlated significantly with cognitive, motor and executive functions (grey matter: $P < 0.05$, white matter: $P < 0.01$). Our findings suggest that there are long-lasting cerebral changes in adolescent survivors of cardiopulmonary bypass surgery for congenital heart disease and that these changes are associated with functional outcome.

Keywords: congenital heart disease; adolescents; cardiopulmonary bypass surgery; brain volume; neurodevelopment

Abbreviation: CHD = congential heart disease

Received May 15, 2013. Revised September 10, 2013. Accepted September 26, 2013.

Introduction

Severe congenital heart disease (CHD) requiring surgical intervention occurs in 6 of 1000 live-born infants (Hoffman and Kaplan, 2002). Although substantial improvements in surgical treatment and perioperative care have led to excellent cardiac outcomes and consecutively a significant increase in survival rates, impairments of neurodevelopmental and behavioural outcome have been described for school age and early adolescence (Bellinger *et al.*, 2003, 2011; von Rhein *et al.*, 2011). The population of adolescent and adult patients with CHD is increasing, and it is therefore of importance to identify potential non-cardiac sequelae in this population.

The high rate of neurodevelopmental impairments in patients with CHD, including cognitive and motor deficits, and difficulties with daily living skills, communication or adaptive behaviour (Bellinger *et al.*, 2003; Hovels-Gurich *et al.*, 2006; Majnemer *et al.*, 2008; Snookes *et al.*, 2010; von Rhein *et al.*, 2011) has prompted foetal and neonatal cerebral neuroimaging studies to elucidate the underlying causes of brain injury. They have demonstrated delayed brain maturation in infants with severe CHD, featuring delays in myelination and cortical folding as well as abnormal brain metabolism and microstructure (Miller *et al.*, 2007; Licht *et al.*, 2009; Limperopoulos *et al.*, 2010). In addition, preoperative brain injury, consisting mostly of white matter changes, can be seen in up to 50% of patients (Mahle *et al.*, 2002; McQuillen *et al.*, 2007; Miller *et al.*, 2007). New postoperative lesions comparable to the ones seen in preterm-born infants have been described in ~30% of infants in a regional distribution (Mahle *et al.*, 2002; McQuillen *et al.*, 2007). Recently, structural brain lesions have also been reported in adolescents after cardiopulmonary bypass surgery for CHD (Bellinger *et al.*, 2011; von Rhein *et al.*, 2011), showing predominantly focal white matter abnormalities and white matter volume reduction.

Importantly, the relationship between neuroimaging findings and neurodevelopmental impairments has not yet been established. Brain volume reduction has been quantified for young children with CHD (Watanabe *et al.*, 2009), and a weak relationship has been found between frontal grey matter volume and early motor development (Watanabe *et al.*, 2009). On the other hand, a variety of studies demonstrated regional brain volume reductions in the cerebellum, hippocampus and corpus callosum, which were associated with cognitive and perceptual functions in former preterm-born adolescents (Martinussen *et al.*, 2009; Taylor *et al.*, 2011). Thus, in patients with CHD, the functional relevance of imaging findings in relation to neurodevelopmental outcome needs to be established. As structural MRI findings (Bellinger *et al.*, 2011; von Rhein *et al.*, 2011) have been correlated with functional outcomes in adolescent survivors of CHD, a correlation between total and regional brain volumes and functional outcomes, similarly to findings in preterm adolescents, would be expected. Furthermore, results regarding cyanosis as a risk factor for adverse neurodevelopmental outcome are controversial.

Thus, the aims of our study were to (i) investigate whether brain morphometric measurements (global and regional brain volumes, cortical thickness and surface area) are reduced in adolescents with corrected CHD; (ii) to examine the relationship between quantitative brain measurements and functional outcome. We hypothesized that we would find primarily reduced white matter volumes. We further hypothesized associations between reduced white matter and deficits in cognitive performance; and (iii) we aimed to identify risk factors for volume reductions in adolescent heart patients without overt brain lesions, and specifically examined whether the type of CHD (cyanotic versus acyanotic) was associated with poorer functional and structural outcome.

Materials and methods

Participants

Participants had undergone cardiac surgery for different types of CHD with full flow cardiopulmonary bypass at the University Children's Hospital Zurich between 1995 and 1998 (median age at first surgery: 0.9 years; range 0–5.6 years). Children with a diagnosis of known or suspected chromosomal and genetic syndromes or a neurological comorbidity were excluded (Landolt *et al.*, 2008). Of 117 adolescent patients who took part in a previous follow-up study in 2004–05 (Landolt *et al.*, 2008), we excluded those with an age of 17 years or older at the time of this study, those wearing braces, pacemakers, or cochlear implants, and those who had reported claustrophobia. Twenty-three of the 78 eligible adolescents refused participation, and two were lost to follow-up. Hence, 53 adolescents with CHD (median age 13.8 years, range 11.4–16.9, 46% male) were assessed. Demographic and surgical characteristics were not significantly different between participants and patients lost to follow-up. Nevertheless, the participants of this study represented a subgroup with fewer neurological abnormalities (33% versus 60%, $P < 0.001$) and better cognitive functioning at 10 years of age (IQ at 10 years: 93.2 ± 18.4 versus 86.1 ± 14.1, $P = 0.02$) compared to those lost to follow-up. As described previously, cerebral imaging abnormalities were detected in 11 of 53 patients (21%) and in none of the control subjects (von Rhein *et al.*, 2011). The 11 patients with imaging abnormalities were excluded from the morphometric analyses. Neurodevelopmental outcome of the excluded individuals was poorer than those of patients without brain lesions (von Rhein *et al.*, 2011). In three patients, morphometric analyses could not be performed due to movement artefacts. Therefore, morphometric measures were performed in 39 patients (surface based analysis, see Supplementary Table 2). Results were compared to 32 healthy control subjects (median age 13.9 years, range 9–16.9; 41% male) who either were enrolled for this study ($n = 11$) or who had participated as controls in another study ($n = 21$). All went to regular school and none suffered any chronic or neurological diseases. Controls had no brain lesions visible on MRI. Groups were not significantly different with regards to sex and socioeconomic status and underwent identical neurodevelopmental assessment.

Neurodevelopmental assessment

Neurodevelopmental assessment included a standardized neurological examination, the Zurich Neuromotor Assessment, the Wechsler Intelligence Scale for Children fourth edition, the Beery Test of visuo-motor integration, and the Rey-Osterrieth Complex Figure Test. Neurodevelopmental assessment was performed before the MRI examination by an experienced paediatric neurologist (M.vR) who was aware of the medical condition but not the imaging results.

Image acquisition

Brain MRI was performed with a 3.0 T whole-body system (SignaTwinspeedHD.xt, GE Healthcare). Three-dimensional high resolution anatomical images of the entire brain were obtained using an inversion-recovery prepared T_1-weighted spoiled gradient echo pulse sequence (repetition time = 25 ms; echo time = 5 ms, inversion time = 450 ms, flip angle = 13°, field of view = 24 cm, acquisition matrix = 352 × 224, slice thickness 1.2 mm, image resolution 0.47 × 0.47 mm) and an axial T_2-weighted fast spin echo pulse sequence (repetition time = 6660 ms; echo time = 97.6 ms; field of view = 24 cm, acquisition matrix = 512 × 384, slice thickness 3 mm, image resolution 0.47 × 0.47 mm). Anatomical evaluation was performed by an experienced neuroradiologist (I.S.) who was blinded to the participants' medical history. The 3D T_1-weighted spoiled gradient echo whole brain images were also used for morphometric assessment.

Post-processing

Surface-based analysis

All images were transformed into Analyze format and entered into a standard morphometric analysis using Freesurfer version 4.5.0 for Macintosh computers (http://surfer.nmr.mgh.harvard.edu). This software calculates subcortical volumes and cortical areas and thicknesses. The cortical stream tessellates each hemisphere on the grey–white border and allows the measurement of cortical thicknesses at each point of the tessellation. In addition, the volumes and cortical thicknesses of many individual gyri and sulci can be measured. The total brain volume was calculated as the sum of the grey and white matter volumes of the cortical hemispheres, callosal and subcortical volumes (including cerebellum), and the volumes of the inner and outer CSF compartments. For further statistical analyses, cortical and subcortical volumes were pooled over the left and right hemisphere structures.

Additionally, a voxel-based morphometry analysis was conducted, involving a segmentation into grey matter white matter, CSF and three classes of non-brain tissue with the 'new segment' function. These segmented maps were warped into a common space using a diffeomorphic transformation (Dartel tools implemented in SPM8; http://www.fil.ion.ucl.ac.uk/spm/software/spm8). Then, each native T_1-weighted image was warped using these segmentation maps in an attempt to minimize distortions because of differences of the patient and children's brains to the template and to thereby maximize sensitivity. Initial segmentation was successful in 44 participants. The others had lower image quality and were discarded from further analysis. This complementary analysis allowed visualization of the exact locations of the volumetric changes and detection of finer differences within subcortical structures than those found in the global and surface-based analyses. Sex, age and total brain volumes were always linearly regressed out as control variables of no interest.

Statistics

All behavioural and Freesurfer data were analysed using PASW© statistics software [PASW (SPSS) statistics 18.0, IBM]. Comparisons of study groups were controlled for sex and age at the time of the scan. To determine the associations between brain volume and neurodevelopmental performance, regression analyses with cognitive and motor test scores as dependent variables were conducted for total brain volume and total grey and white matter volumes. To determine the independent association between regional brain volumes and functional outcome, multiple stepwise linear regression models were performed, each including total brain volume and the brain regions that were significantly reduced on bivariate analysis (total cortical grey matter, white matter, volumes of cerebellum, hippocampi and corpus callosum) as well as sex and age at scan. Additionally, we performed stepwise linear regression analyses including total brain volume and the lobal surface areas of the cortical lobes as well as sex and age at scan to investigate any lobe-specific associations with functional outcome. All regression analyses were performed separately for patients and controls. We used *P*-values of 0.05 and 0.10 to enter or remove variables, respectively.

Finally, multiple regression analyses were calculated to identify risk factors for volume reduction in the heart patient group. For each potential risk factor (type of heart disease, cyanosis, duration of heart surgery, extracorporeal circulation time, aortic cross-clamp time, length of intensive care unit stay, length of hospital stay), a separate regression model was performed including sex, age at scan, and total brain volume as further independent variables.

Ethical approval was obtained from the Ethics Committee at the University Children's Hospital and written informed consent was obtained from the parent or primary caregivers and from the adolescents.

Results

Patient characteristics

Medical characteristics of the patient group are provided in Supplementary Table 1. Neurodevelopmental performance was poorer in patients with CHD than in control subjects, reaching statistical significance in 7 of 15 domains (Supplementary Table 2). No significant difference in outcome was found between patients with cyanotic and acyanotic CHD (data not shown). Former preterm-born patients with CHD showed no significant differences in neurodevelopmental outcome and brain volumes compared to former term-born CHD patients (Mann-Whitney U-test, data not shown).

Morphometric results

Patients after surgery for CHD had smaller total brain volumes, total white matter, and cortical and deep grey matter volumes than control subjects, whereas ventricular volumes were not different (Table 1). Total grey matter reduction was more pronounced in the limbic cortex, frontal and parietal lobes, and cerebellum. White matter reduction did not show any specific regional predilection (Fig. 1). *T*-tests for all available cortical lobes showed no significant differences in cortical thickness between patients and controls. Group differences in regional volumes mostly disappeared when correcting for total brain volume (data not shown). We found a significant positive correlation between white matter volume and age at scan (beta = 0.30, $P < 0.001$, controlled for total brain volume) in the CHD patient group, but not in the control group (Fig. 2). Total brain volume and grey matter volumes did not correlate significantly with age in either group. Cortical thickness was negatively correlated with age in the congenital heart patient group (mean thickness of all sulci and gyri: $R^2 = -0.52$, $P = 0.001$, prefrontal cortices: $R^2 = -0.50$, $P = 0.001$, occipital lobes: $R^2 = -0.39$, $P = 0.02$), whereas no such correlations were found in the control group.

Table 1 Global and regional brain volumes in patients and controls

	Controls, $n = 32$ mean (SD)	Patients, $n = 39$ mean (SD)	% Reduction
Total brain volume	1216 (112)	1130 (120)	7.0*
White matter volume	434 (47)	397 (55)	8.5**
Cortical grey matter volume	570 (63)	532 (51)	5.3**
Ventricular volume	13.23 (4.91)	13.85 (6.29)	
Volume gyri total	379 (41)	354 (36)	6.6**
Volume sulci total	190 (22)	174 (19)	8.4**
Surface area gyri total	1165 (129)	1078 (115)	7.5**
Surface area sulci total	828 (96)	765 (90)	7.6**
Thickness gyri total	2.69 (0.11)	2.69 (0.10)	0
Thickness sulci total	2.40 (0.09)	2.41 (0.07)	0
Surface area frontal lobes	351 (49.7)	326 (39.7)	7.1*
Surface area temporal lobes	327 (46.2)	301 (38.8)	7.9**
Surface area parietal lobes	216 (24.4)	202 (24.0)	6.5**
Surface area occipital lobes	83 (12.9)	78 (9.5)	6
Surface area limbic cortices/ mesocortices	111 (12.4)	100 (9.9)	9.9***
Volume cerebellum	145 (14.1)	136 (12.2)	6.2**
Volume thalami	14.4 (1.2)	13.7 (1.6)	4.9*
Volume basal ganglia	24.5 (1.8)	22.8 (2.5)	6.9**
Volume hippocampi	8.2 (0.8)	7.5 (0.7)	8.5***
Volume corpus callosum	3.09 (0.39)	2.75 (0.47)	11.0***

Results of surface-based morphometry, reduction in % (controls = 100%), *P*: *t*-test for means, brain volumes in cm^3, surface areas in cm^2, thickness in mm; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

In a separate analysis, we compared brain volumes of patients in our cohort with those patients with overt cerebral lesions who had been excluded from the main analyses. No significant differences were detected between these groups.

Risk factors

All brain volumes were smaller in patients with cyanotic CHD than in those with acyanotic CHD, reaching significant levels ($P < 0.05$) for white matter, thalami, hippocampi and corpus callosum volumes. Furthermore, patients with acyanotic heart disease had smaller cortical grey matter, cerebellum, basal ganglia and hippocampal volumes than control subjects (Table 2). Stepwise logistic regression analyses showed that cyanotic CHD was an independent predictor of callosal (beta = −0.35, $P = 0.01$) and hippocampal size (beta = −0.21, $P = 0.04$) in patients, even when entering total brain volume, age and sex (corpus callosum: beta = 0.38, $P = 0.006$, hippocampi: beta = 0.71, $P < 0.001$, respectively) into the equation. No other risk factor (socioeconomic status, duration or number of surgeries, length of hospital stay, or postoperative EEG or neurological abnormalities) correlated with morphometric measures.

Correlation between brain volumes and functional outcome

Figure 3 shows the significant correlations between total brain volume and IQ in patients with CHD. Significant correlations were also found between total brain volume and other cognitive function measures (verbal comprehension: beta = 0.41, $P = 0.01$, perceptual reasoning: beta = 0.36, $P = 0.04$, working memory: beta = 0.34, $P = 0.04$) and static balance (beta = 0.36, $P = 0.03$) independent of disease severity (cyanotic or acyanotic). In control subjects, no association was found between total brain volume and functional outcome.

When analysing the impact of specific brain regions on neurodevelopmental outcome within the patient group, stepwise regression analyses (adjusted for socioeconomic status, sex, age at assessment and total brain volume) showed that hippocampal volume was related to total IQ and perceptual reasoning. Total white matter volume correlated with verbal comprehension and motor performance, and cerebellar volume correlated with working memory and static balance (Table 3). In the control group, hippocampal volume correlated with perceptual reasoning index ($R^2 = 0.12$, beta = 0.38, $P = 0.04$), corpus callosum volume with processing speed ($R^2 = 0.20$, beta = −0.48, $P = 0.007$), and cerebellar volume with fine motor performance ($R^2 = 0.16$, beta = 0.44, $P = 0.02$). No significant correlation with functional outcome was found with respect to the surface areas of the cortical lobes. Head circumference was smaller in patients [53.4 cm, standard deviation (SD) 2.0] than in control subjects (55.6 cm, SD 1.5; $P = 0.01$), but did not correlate with neurodevelopmental outcome in either group.

Discussion

In our population of adolescents operated for CHD without overt structural brain lesions, we found a global brain volume reduction affecting both grey and white matter. Further, there was no region-specific volume reduction after correction for total brain volume. Brain volumes correlated with functional outcome in adolescents with CHD, but not in control subjects. Cyanotic CHD was a risk factor for smaller brain volumes. Importantly, functionally relevant brain volume reductions were also detected in patients without overt structural brain abnormalities. This highlights the importance of quantitative imaging measurements in this population. As a significant number of patients with CHD show brain alterations at least until adolescence, this population needs not only long-term specialized cardiac care but also structured neuropsychological assessments.

Distribution of brain volume reduction

We previously reported structural imaging findings for this patient cohort, which included white matter lesions and signs of previous arterial stroke (von Rhein *et al.*, 2011). We could confirm our hypothesis that brain volumes were significantly reduced in patients without overt lesions compared with control subjects. The white matter volume was particularly affected, which corresponds to the predominant white matter structural injury pattern in adolescents with CHD (Bellinger *et al.*, 2011; von Rhein *et al.*, 2011). In addition, neonatal magnetic resonance studies have shown alterations predominantly in the white matter, similar to the injury pattern found in preterm-born infants (Childs *et al.*, 2001; Miller

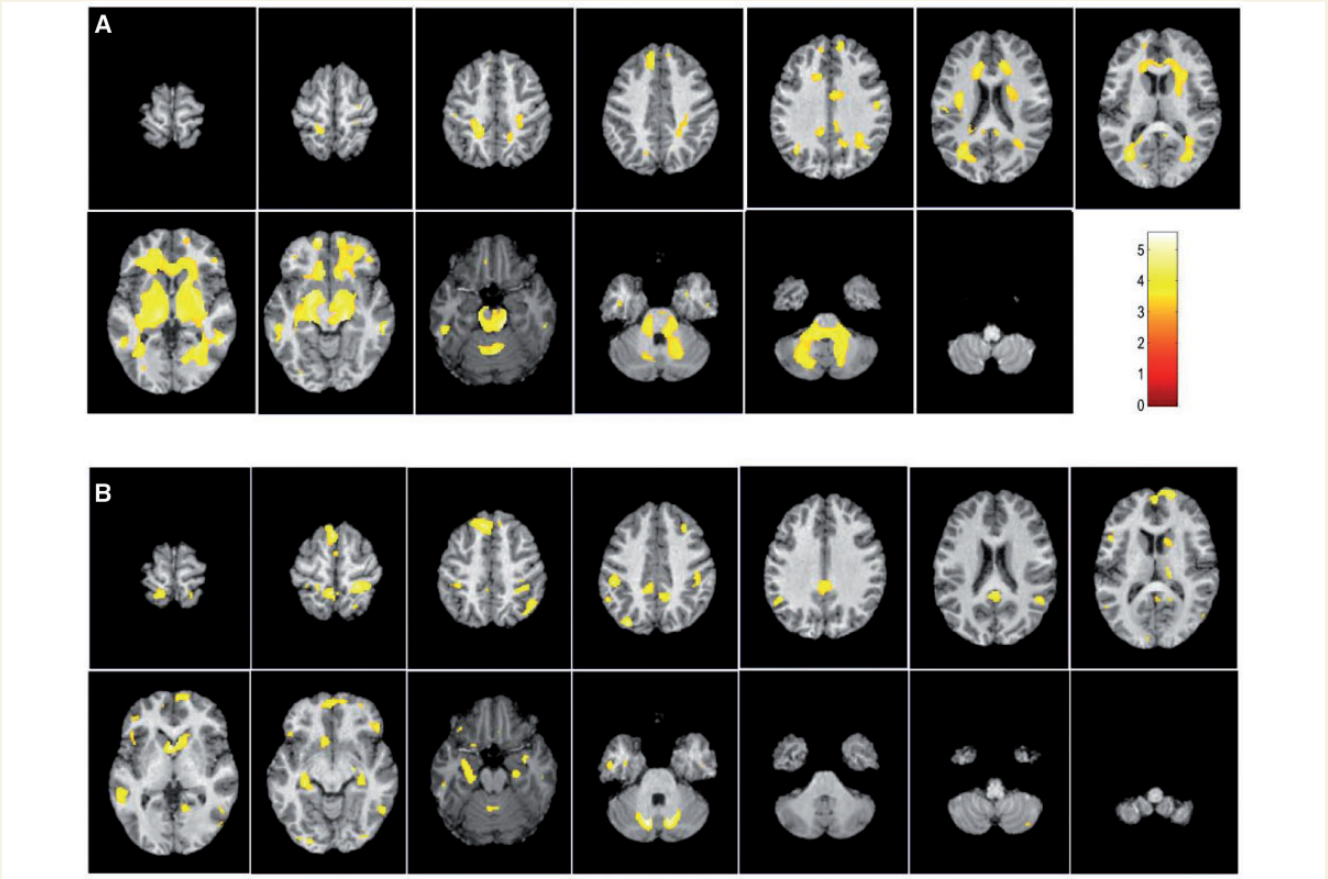

Figure 1 Colour brain maps (axial plains) expressing the differences between the regional brain volumes of patients with CHD and controls; $P < 0.001$. Colours represent *T*-values (corrected for age and sex). (**A**) White matter; (**B**) grey matter.

Table 2 Comparison of brain volumes between patients with acyanotic and cyanotic CHD and control subjects

	Controls $n = 32$ Mean (SD)	Acyanotic heart disease $n = 20$ Mean (SD)	*P*-value: controls versus acyanotic	Cyanotic heart disease $n = 19$ Mean (SD)	*P*-value: acyanotic versus cyanotic
Total brain volume	1216 (112)	1168 (125)		1108 (101)	
White matter volume	434 (47)	418 (63)		377 (47)	<0.05
Cortical grey matter volume	570 (63)	539 (58)	<0.05	526 (46)	
Ventricular volume	13.23 (4.91)	13.10 (4.48)		14,35 (6.75)	
Surface area gyri total	1165 (129)	1115 (116)		1059 (104)	
Surface area sulci total	828 (96)	798 (95)		744 (69)	
Thickness gyri total	2.69 (0.10)	2.68 (0.11)		2.72 (0.12)	
Thickness sulci total	2.41 (0.07)	2.39 (0.09)		2.42 (0.09)	
Surface area frontal lobes	351 (50)	337 (44)		322 (34)	
Surface area temporal lobes	327 (46)	316 (45)		284 (30)	<0.05
Surface area parietal lobes	216 (24)	208 (23)		194 (24)	
Surface area occipital lobes	83 (13)	79 (9)		77 (9)	
Surface area limbic cortices/ mesocortices	111 (12.4)	102 (11)	<0.01	98 (9)	
Volume cerebellum	145 (14.1)	137 (13.1)	<0.01	136 (11.4)	
Volume thalami	14.4 (1.2)	14.3 (1.7)		13.0 (1.2)	<0.05
Volume basal ganglia	24.5 (1.8)	23.1 (2.5)	<0.05	23.0 (2.4)	
Volume hippocampi	8.2 (0.8)	7.9 (0.8)	<0.05	7.2 (0.6)	<0.05
Volume corpus callosum	3.09 (0.39)	2.97 (0.29)		2.56 (0.41)	<0.01

Results of surface-based morphometry, *P*: *t*-test for means of controls and acyanotic, and acyanotic versus cyanotic heart disease: brain volumes in cm^3, surface areas in cm^2, thickness in mm.

et al., 2007; Licht *et al.*, 2009; Limperopoulos *et al.*, 2010). Only one study so far has demonstrated grey matter volume reduction at the age of 15 months (Watanabe *et al.*, 2009). The combination of grey and white matter volume reductions in our sample may reflect a combination of the late sequelae of delayed intrauterine brain maturation, as well as acquired perioperative brain injury. In contrast to the global volume changes in our cohort, (Ortinau *et al.*, 2012*a*, *b*) found a more regional brain size reduction in infants with CHD before surgery, but a normal brain growth at 3 months of age after surgical repair. These findings could indicate an undisturbed myelination at this early age. Interestingly, in our sample, patients with CHD showed a positive correlation between age and total white matter volume. This may suggest an ongoing white matter development in patients that could not be detected in controls despite similar ages at scanning. These findings may indicate that patients with CHD undergo a longer maturation period because of a delay in white matter maturation. Longitudinal data would be needed to prove this hypothesis.

We found a normal cortical thickness but reduced cortical surface areas, which was roughly proportional to the number of cortical columns and closely related to the number of radial glia. Assuming a constant number of connections for each cortical column, this would imply a strongly reduced number of axons, which could explain the widespread reduction in white matter volume. Conversely, a reduced cortical surface could also be a result of axonal loss due to white matter injury or delayed development. We mainly found reduced grey matter volumes in patients with acyanotic CHD compared with controls, although cyanotic patients also showed a decrease in white matter volume. This observation accounts for both superficial and deep grey and white matter structures. It seems logical that the thalami, which also contain white matter, follow the pattern of the cortical white matter and corpus callosum in this respect. The subcortical cores, which interact closely with the cortical columns, show a reduction that resembles the reduction of the cortical surface in effect size. Studies in preterm neonates have shown subcortical changes in this population, which have been attributed to impaired oxygen supply. Subcortical regions and white matter are generally considered important for cognitive and motor functions, so the widespread distribution of volume differences in our patients with CHD could lead to a functional sum effect of all regions involved (cortical and subcortical regions and white matter). In patients with CHD, delayed brain maturation at term, along

Table 3 Reduced brain volumes in association with neurodevelopmental outcome in patients

	Predictor	Corrected R^2	Beta	*P*-value
Cognitive abilities				
Total IQ	Hippocampal volume	0.18	0.45	0.004
Verbal comprehension	Total white matter	0.17	0.44	0.006
Perceptual reasoning	Hippocampal volume	0.11	0.36	0.03
Working memory	Cerebellar volume	0.16	0.42	0.008
Processing speed				
Motor abilities				
Pure motor	Total white matter	0.15	0.41	0.01
Adaptive fine motor				
Dynamic balance				
Static balance	Cerebellar volume	0.11	0.36	0.03

Stepwise linear regression analyses (dependent variable: functional outcomes). Variables included in each model: volumes of total brain tissue, white matter, cortical grey matter, cerebellum, corpus callosum, and hippocampus, sex and age at scan.

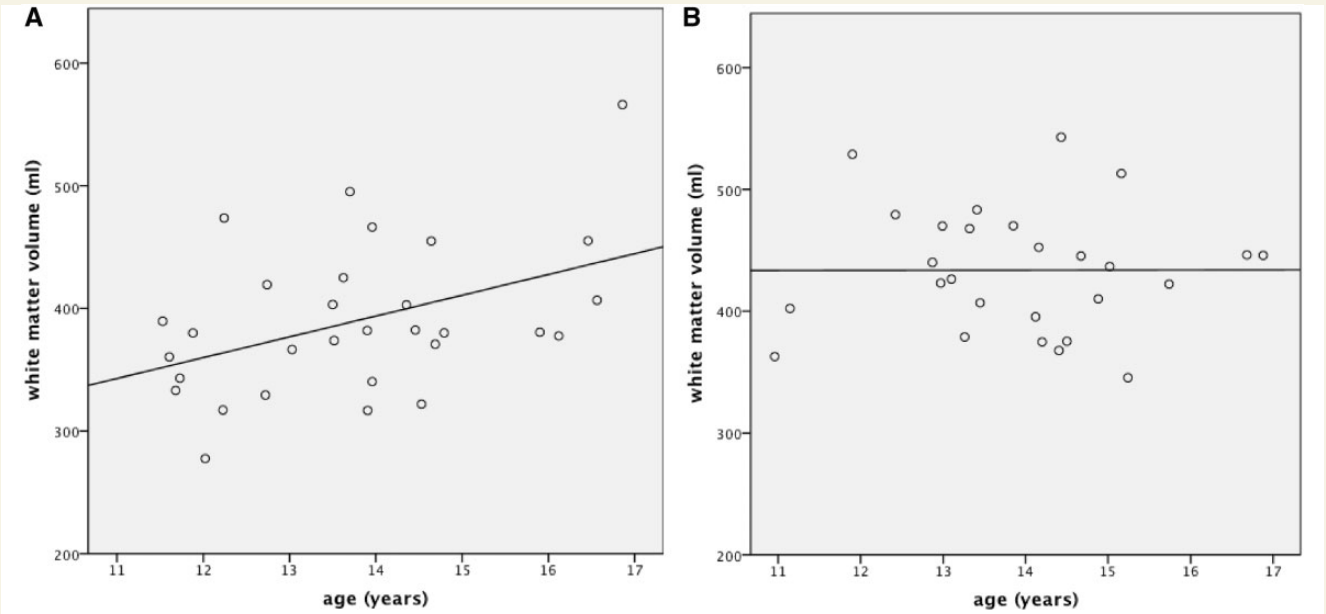

Figure 2 Scatterplots of white matter volumes plotted versus age (separately for patients and controls). Linear regression curves of (**A**) patients ($R^2 = 0.36$, $P = 0.05$) and (**B**) controls ($R^2 = -0.05$, $P = 0.82$).

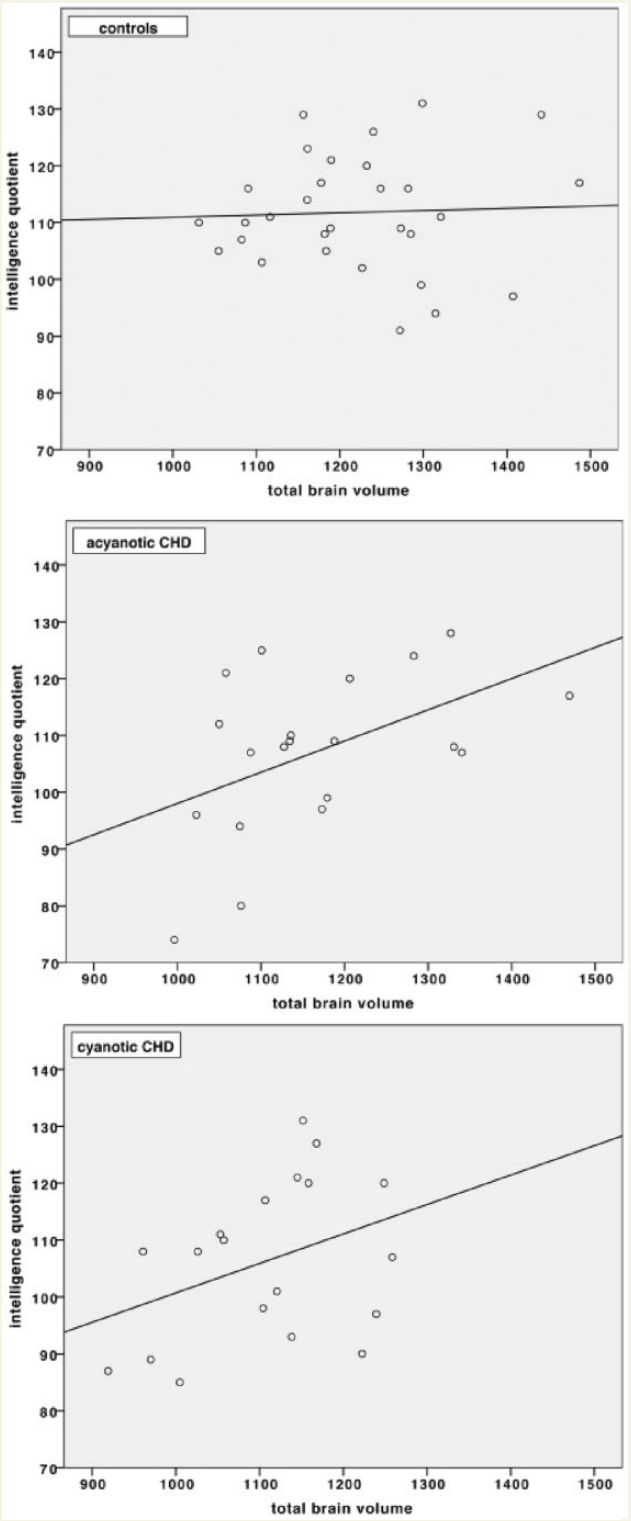

Figure 3 Scatterplot of intelligence quotient versus total brain volume (no CSF), plotted separately for controls and acyanotic and cyanotic patients. Linear regression curves of controls ($R^2 = 0.002$), acyanotic ($R^2 = 0.233$) and cyanotic heart disease ($R^2 = 0.138$).

with early acquired white matter lesions secondary to cardiopulmonary bypass surgery, could cause further alterations in brain development with impacts on both white and grey matter structures, leading to the widespread brain volume reduction observed in our adolescent patients. This mechanism has been shown by neuroimaging studies in former preterm-born adolescents (de Kieviet *et al.*, 2012).

Interestingly, brain volumes in the excluded patients with cerebral lesions were not different from those without overt lesions. This suggests mechanisms that independently involve the entire brain in addition to the focal injuries observed in perioperative imaging studies (Miller *et al.*, 2007; Licht *et al.*, 2009; Limperopoulos *et al.*, 2010,). We therefore interpret our findings as the joint global result of delayed intrauterine brain development, early brain injury and consecutively altered brain development over a prolonged period of time. Our findings highlight the importance of quantitative MRI measurements, as qualitative visual neuroradiological assessment seems to underestimate the extent of brain volume loss.

Brain volume reduction affects neurodevelopmental outcome

The brain volume reduction observed in adolescent patients with CHD is of clinical relevance, as global and regional brain volumes correlated with neurodevelopmental functioning (Fig. 2 and Table 3). This association is even more remarkable as patients with overt lesions and worse neurodevelopmental outcome (von Rhein *et al.*, 2011) were excluded from our analyses. As hypothesized, we showed an association between reduced total brain volume and white matter volume and deficits in cognitive performance. Furthermore, we found correlations between regional volumes (i.e. corpus callosum, hippocampus and cerebellar volume) and cognitive abilities in our patients with CHD, similar to studies in former preterm-born adolescents (Martinussen *et al.*, 2009; Northam *et al.*, 2011).

The strong association between white matter reduction and outcome is in agreement with diffusion-weighted imaging and functional MRI studies that have shown parameters of long-range connectivity to correlate with working memory (Nagy *et al.*, 2004), intelligence (Schmithorst *et al.*, 2005), and language skills across development in normally developing children and adolescents. The loss of white matter could be (perhaps non-linearly) associated with less connected and thus less efficient neuronal networks in the CHD patient group. If so, there might only be a weak association between brain volume and intelligence as long as white matter volume does not fall below a certain limit. However, an intrauterine delay in myelination (Miller *et al.*, 2007; Licht *et al.*, 2009) could exacerbate functional impairments by a reduction of synapses formation.

It would have been interesting to compare our results with functional MRI data. As such measurements were not part of our study, the regional distribution of correlating areas should be interpreted cautiously. We assume they rather represent the areas of the most prominent volume loss, and therefore show the strongest correlations with functional outcomes. This does not necessarily imply that they are functionally more relevant than other regions. Nevertheless, we regard the regions associated with

poor outcome in our study to be the indicators of a variety of functionally relevant brain areas. This is also supported by the fact that in the control group the association between total brain volume and overall IQ was not present, and fewer correlations between regional volumes and functional performance were found. The fact that a significant relationship between structural and functional findings were only detected in patients, but not in controls, could have been because of a greater variability in the volumes and neurodevelopment outcomes of the patients, thereby leading to a greater power to detect a correlation. However, we did not find such a difference: structural and functional results were equally distributed in controls and patients (Fig. 2). Therefore, it is conceivable that reduced brain volumes lead to an impaired function, but it could also be that a common, third factor could lead to both reduced functional and structural outcomes, e.g. altered and delayed intrauterine brain development, as has been shown by Miller *et al.* (2007).

Risk factors

In our study, adolescents with cyanotic CHD had the most pronounced brain volume reductions. Adolescents with an acyanotic CHD also showed a significant volume reduction compared with control subjects; this was, however, less pronounced than in those with cyanotic CHD. This finding broadens current knowledge of how cardiac disease severity affects brain development and injury. Surprisingly though, in contrast with the morphometric findings, there was no difference in neurodevelopmental outcome between patients with cyanotic and acyanotic heart disease. This may be explained by the fact that environmental factors and compensatory pathways may lead to similar functional results despite reduced brain volumes. Another explanation might be that patients with cyanotic heart disease can rely on more localized networks instead of distributed networks, with comparable functional outcome despite smaller brain volumes.

Besides CHD severity, no other independent clinical risk factor predicted morphometric measures. This contrasts with a recent publication reporting total bypass time, length of hospital stay, and catheterization exposure to be significantly correlated with structural abnormalities on MRI in adolescent patients with CHD (Bellinger *et al.*, 2011). Presumably, this depends greatly on the variability of the patient sample and surgical methods applied. For example, our patients had a variety of different cardiac diagnoses and were operated at an older age than the cohort reported by Bellinger *et al.* (2011), which consisted only of patients with a d-transposition of the great arteries undergoing either deep hypothermic cardiopulmonary arrest or low-flow cardiopulmonary support.

Future perspective

Sixty years after the first successful cardiopulmonary bypass surgery in an 18-year-old young adult, the surgical closure of an atrial septal defect, a new patient population of adults with CHD has evolved. Overall mortality has decreased to $<5\%$ in most centres as a result of advances in the treatment of CHD, leading to a continuously rising number of patients reaching adulthood. Despite the low mortality rate, the most complex types of cyanotic heart disease are still challenging, not only with respect to their cardiological and cardio-surgical treatment, but also regarding their long-term follow-up until adulthood: presumably, adults with CHD are at risk of a wide range of neurodevelopmental impairments with cognitive and motor deficits, and many have difficulties with daily living skills, communication or adaptive behaviour, as has been shown for adolescents with CHD (Bellinger *et al.*, 2003; Hovels-Gurich *et al.*, 2006; Ballweg *et al.*, 2007; Majnemer *et al.*, 2008; Snookes *et al.*, 2010; von Rhein *et al.*, 2011). Therefore, adults with CHD require a multidimensional medical attendance, which has to be provided not only by paediatric and adult cardiologists, but also by neurologists and neuropsychologists.

Limitations

Several limitations of our study have to be mentioned. Our cohort does not include one single diagnosis of CHD but covers a wide spectrum of diagnoses. Because of the rather small sample size, conclusions for individual types of CHD cannot be drawn. Furthermore, children with hypoplastic left heart syndrome were not surgically treated in our institution in the late 1990s and thus could not be included in this cohort. Therefore, our results most likely underestimate the extent of long-term cerebral changes in the population with CHD currently being treated. Furthermore, we were not able to compare adolescent cerebral imaging findings to neonatal imaging or preoperative clinical findings, thus limiting the conclusions on timing of brain injury and intrauterine brain growth.

Conclusion

We found a significant reduction of global and regional brain volume in adolescent CHD patients without overt brain lesions compared to healthy controls. Brain volume reduction was more pronounced in patients with cyanotic heart disease. It correlated significantly with neurodevelopmental performance, highlighting the relevance of these widespread cortical and subcortical volume reductions for cognitive outcome. Our findings provide important new contributions to the understanding of the long-term effects of brain injury in patients with CHD. Delayed brain maturation at term and neonatal brain injury with its consequences on brain development seem to persist into adolescence and are functionally significant. Anatomically detected brain lesions seem to be only the tip of the iceberg, and even subtle brain volume reduction can be functionally relevant.

Acknowledgements

We thank Ianina Scheer for assessment of the images and neuroradiologic counselling, and Ruth O'Gorman for reviewing an earlier draft of this paper.

Funding

Swiss Heart Foundation, Else Kröner-Fresenius-Foundation. CH was supported by the Swiss National Foundation Marie Heim-Vögtlin Grant PMPDP3_129104. The sponsors had no influence on study design, the collection, analysis, and interpretation of data, the writing of the report, or the decision to submit the paper for publication. The manuscript has been written by the first authors.

Supplementary material

Supplementary material is available at *Brain* online.

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